NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*DNA Polymerase I, Large (Klenow) Fragment*
Catalog #:	*M0210S/L*
Concentration:	*5,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.*
Lot #:	*0891709*
Assay Date:	*09/2017*
Expiration Date:	*9/2019*
Storage Temp:	*-20°C*
Storage Conditions:	*25 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0210S/L v1.0*
Effective Date:	*16 Oct 2015*

Assay Name/Specification (minimum release criteria)	**Lot #0891709**
Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 2 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of DNA Polymerase I, Large (Klenow) Fragment incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units DNA Polymerase I, Large (Klenow) Fragment incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	**Pass**
Protein Purity Assay (SDS-PAGE) - DNA Polymerase I, Large (Klenow) Fragment is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 50 units of DNA Polymerase I, Large (Klenow) Fragment is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.	**Pass**
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of DNA Polymerase I, Large (Klenow) Fragment is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**

Authorized by
Melanie Fortier
16 Oct 2015

Inspected by
David Guo
28 Aug 2017

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices